

Serial No.: 09/116,502  
Docket No.: CL1035

Page 2

4. The transformed *Pichia pastoris* of Claim 3 wherein the foreign gene encoding cytochrome P450 monooxygenase is selected from the group consisting of Alk1-A (D12475 (SEQ ID NO:35)), Alk2-A (X55881 (SEQ ID NO:36)), Alk3-A (X55881 (SEQ ID NO:37)), Alk4-A (D12716 (SEQ ID NO:38)), Alk5-A (D12717 (SEQ ID NO:39)), Alk6-A (D12718 (SEQ ID NO:40)), Alk7 (D12719 (SEQ ID NO:41)), and Alk8 (D12719 (SEQ ID NO:42)).

5. The transformed *Pichia pastoris* of Claim 3 wherein the foreign gene encoding cytochrome P450 reductase is cytochrome P450 reductase (D25327 (SEQ ID NO:43)).

6. A transformed *Pichia pastoris* strain comprising an enhanced alkane hydroxylating activity and comprising,

a) at least one DNA fragment from *Candida maltosa* ATCC 90677 selected from the group of DNA fragments encoding cytochrome P450 monooxygenase Alk1-A (SEQ ID NO:35) and cytochrome P450 monooxygenase Alk3-A (SEQ ID NO:37); and, optionally,

b) at least one DNA fragment from *Candida maltosa* ATCC 90677 encoding cytochrome P450 reductase,  
each DNA fragment operably linked to suitable regulatory elements such that alkane hydroxylating activity is enhanced upon contact with at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon.

7. A transformed *Pichia pastoris* strain SW64/65 identified as ATCC 74409.

8. A method for the enhanced bioproduction of C<sub>6</sub> to C<sub>22</sub> mono- and di-carboxylic acids comprising

a) contacting, under aerobic conditions, a transformed *Candida maltosa* comprising a genetically-engineered, enhanced alkane hydroxylating activity with at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon, wherein said alkane hydroxylating activity arises from

i) at least one additional copy of the genes encoding cytochrome P450 monooxygenase selected from the group consisting of Alk1-A (D12475 (SEQ ID NO:35)), Alk2-A (X55881 (SEQ ID NO:36)), Alk3-A (X55881 (SEQ ID NO:37)), Alk4-A (D12716 (SEQ ID NO:38)), Alk5-A (D12717 (SEQ ID NO:39)), Alk6-A (D12718 (SEQ ID NO:40)), Alk7 (D12719 (SEQ ID NO:41)), and Alk8 (D12719 (SEQ ID NO:42)); or

ii) at least one additional copy of the gene encoding cytochrome P450 reductase (D25327); or

iii) at least one additional copy of both the genes of i) and ii); and

b) recovering the C<sub>6</sub> to C<sub>22</sub> mono- and di-carboxylic acids.

9. The method of Claim 8 wherein

a) the at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon is dodecane; and

b) the product recovered is dodecanedioic acid.

10. A transformed *Candida maltosa* comprising

a) at least one additional copy of an integrated gene encoding cytochrome P450 monooxygenase; or

Serial No.: 09/116,502

Docket No.: CL1035

Page 3

b) at least one additional copy of an integrated gene encoding cytochrome P450 reductase; or

c) at least one additional copy of both the integrated gene encoding P450 monooxygenase and the integrated gene encoding cytochrome P450 reductase, each integrated gene operably linked to suitable regulatory elements such that alkane hydroxylating activity is enhanced upon contact with at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon.

11. The transformed *Candida maltosa* of Claim 10 wherein the genes encoding cytochrome P450 monooxygenase are selected from the group consisting of Alk1-A (D12475 (SEQ ID NO:35)), Alk2-A (X55881 (SEQ ID NO:36)), Alk3-A (X55881 (SEQ ID NO:37)), Alk4-A (D12716 (SEQ ID NO:38)), Alk5-A (D12717 (SEQ ID NO:39)), Alk6-A (D12718 (SEQ ID NO:40)), Alk7 (D12719 (SEQ ID NO:41)), and Alk8 (D12719 (SEQ ID NO:42)).

12. The transformed *Candida maltosa* of Claim 10 wherein the gene encoding cytochrome P450 reductase is cytochrome P450 reductase (D25327 (SEQ ID NO:43)).

13. A transformed *Candida maltosa* strain comprising

a) at least one DNA fragment from *Candida maltosa* (ATCC 90677) selected from the group of DNA fragments encoding cytochrome P450 monooxygenase Alk1-A (SEQ ID NO:35) and cytochrome P450 monooxygenase Alk3-A (SEQ ID NO:37), and

b) at least one DNA fragment from *Candida maltosa* (ATCC 90677) encoding cytochrome P450 reductase, each gene operably linked to suitable regulatory elements such that alkane hydroxylating activity is enhanced upon contact with at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon.

14. A method for the enhanced bioproduction of C<sub>6</sub> to C<sub>22</sub> mono- and di-carboxylic acids comprising

a) contacting, under aerobic conditions, transformed *Candida maltosa* comprising a genetically-engineered, blocked  $\beta$ -oxidation pathway with at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon, wherein the  $\beta$ -oxidation pathway is functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase; and

b) recovering the C<sub>6</sub> to C<sub>22</sub> mono- and di-carboxylic acids.

15. Please delete.

16. A transformed *Candida maltosa* comprising disruption of no more than both POX4 genes encoding acyl-CoA oxidase whereby a  $\beta$ -oxidation pathway is functionally blocked.

17. A transformed *Candida maltosa* comprising a  $\beta$ -oxidation pathway functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase using a single URA3 selectable marker.

18. A transformed *Candida maltosa* strain SW81/82 identified as ATCC 74431.

Serial No.: 09/116,502

Docket No.: CL1035

Page 4

19. A method for the enhanced bioproduction of C<sub>6</sub> to C<sub>22</sub> mono- and di-carboxylic acids comprising

a) contacting, under aerobic conditions, transformed *Candida maltosa* comprising,

i) a genetically-engineered, enhanced alkane hydroxylating activity, wherein the enhanced alkane hydroxylating activity arises from

1) at least one additional copy of a gene encoding cytochrome P450 monooxygenase selected from the group consisting of Alk1-A (D12475 (SEQ ID NO:35)), Alk2-A (X55881 (SEQ ID NO:36)), Alk3-A (X55881 (SEQ ID NO:37)), Alk4-A (D12716 (SEQ ID NO:38)), Alk5-A (D12717 (SEQ ID NO:39)), Alk6-A (D12718 (SEQ ID NO:40)), Alk7 (D12719 (SEQ ID NO:41)), and Alk8 (D12719 (SEQ ID NO:42)), or

2) at least one additional copy of a gene encoding cytochrome P450 reductase (D25327 (SEQ ID NO:43)), or

3) at least one additional copy of both the genes i) and ii), and

ii) a genetically-engineered, blocked  $\beta$ -oxidation pathway, wherein the  $\beta$ -oxidation pathway is functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase; and

b) recovering the C<sub>6</sub> to C<sub>22</sub> mono- and di-carboxylic acids.

20. A transformed *Candida maltosa* characterized by

a) an enhanced alkane hydroxylating activity arising from

i) at least one additional copy of a gene encoding cytochrome P450 monooxygenase selected from the group consisting of Alk1-A (D12475 (SEQ ID NO:35)), Alk2-A (X55881 (SEQ ID NO:36)), Alk3-A (X55881 (SEQ ID NO:37)), Alk4-A (D12716 (SEQ ID NO:38)), Alk5-A (D12717 (SEQ ID NO:39)), Alk6-A (D12718 (SEQ ID NO:40)), Alk7 (D12719 (SEQ ID NO:41)), and Alk8 (D12719 (SEQ ID NO:42)), or

ii) at least one additional copy of a gene encoding cytochrome P450 reductase (D25327 (SEQ ID NO:43)), or

iii) at least one additional copy of both the genes i) and ii); and

b) a  $\beta$ -oxidation pathway functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase.

21. The transformed *Candida maltosa* strain of Claim 20 wherein the enhanced alkane hydroxylating activity of a) arises from DNA fragments encoding cytochrome P450 monooxygenase Alk1-A (SEQ ID NO:35) and cytochrome P450 monooxygenase Alk3-A (SEQ ID NO:37).

22. A transformed *Candida maltosa* strain SW84/87.2 identified as ATCC 74430.

23. The method of Claims 1, 8, 14, or 19 wherein the at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon is selected from the group consisting of hexane, heptane, octane, nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, heptadecane,

Serial No.: 09/116,502  
Docket No.: CL1035

Page 5

octadecane, nonadecane, eicosane, reneicosane, docosane and their respective mono-carboxylic acids and esters.

25. An isolated DNA fragment comprising a) a first *Candida maltosa* promoter operably linked to a gene encoding a *Candida maltosa* cytochrome P450 monooxygenase and b) a second *Candida maltosa* promoter operably linked to a gene encoding a *Candida maltosa* cytochrome P450 reductase.

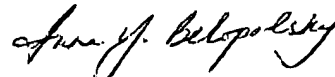
26. An isolated DNA fragment comprising a) a first *Candida maltosa* PGK promoter which is operably linked to a gene encoding cytochrome P450 monooxygenase selected from the group consisting of Alk1-A (D12475 (SEQ ID NO:35)), Alk2-A (X55881 (SEQ ID NO:36)), Alk3-A (X55881 (SEQ ID NO:37)), Alk4-A (D12716 (SEQ ID NO:38)), Alk5-A (D12717 (SEQ ID NO:39)), Alk6-A (D12718 (SEQ ID NO:40)), Alk7 (D12719 (SEQ ID NO:41)), and Alk8 (D12719 (SEQ ID NO:42)) and b) a second *Candida maltosa* PGK promoter operably linked to a gene encoding a *Candida maltosa* cytochrome P450 reductase.

27. A plasmid selected from the group consisting of pSW84 and pSW87.28.

#### REMARKS

In view of the foregoing amendments, allowance of the above-referenced application is respectfully requested.

Respectfully submitted,



INNA Y. BELOPOLSKY  
ATTORNEY FOR APPLICANTS  
REGISTRATION NO. 43,319  
TELEPHONE: (302) 992-4406  
FACSIMILE: (302) 892-7949

Dated: October 8, 2001